

## EAST Search History

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L2	457	436/79.ccls.	US-PGPUB; USPAT; USOCR	OR	ON	2008/01/01 18:33
L3	394	436/79.ccls.	US-PGPUB; USPAT	OR	ON	2008/01/01 18:33
L4	368	436/79.ccls.	USPAT	OR	ON	2008/01/01 18:34
L5	11	bapta and "544".clas.	USPAT	OR	ON	2008/01/01 18:44
L6	22	bapta and "546".clas.	USPAT	OR	ON	2008/01/01 18:44

=> d his

(FILE 'HOME' ENTERED AT 09:56:09 ON 01 JAN 2008)  
FILE 'CA' ENTERED AT 09:56:15 ON 01 JAN 2008  
L1 6982 S (NEAR OR ADJACENT OR PROXIM?) (3A) MEMBRANE  
L2 185032 S (CALCIUM OR CA OR CA2 OR MAGNESIUM OR MG2 OR INDICATOR OR  
FLUOROCHRO? OR FLUOROPHOR?) (5A) (DETECT? OR DETERMIN? OR REPORT?  
OR OPERAT? OR TEST? OR ANALY? OR ASSAY? OR MEASUR? OR MONITOR? OR  
SENSE# OR SENSOR OR SENSING OR PROBE# OR PROBING OR QUANTITAT? OR  
QUANTIF? OR QUANTA?)  
L3 16 S L1(4A) (INDICATOR OR FLUOROPHOR? OR FLUOROCHRO?)  
L4 218 S L1 AND L2  
L5 34 S L1 AND (PIPERAZ? OR ZWITTER?)  
L6 179 S L4 AND PY<2004  
L7 43 S L1(8A) L2  
L8 6 S L6 AND FFP?  
L9 16 S L4/TI, IT, ST  
L10 87 S L3, L5, L7-9  
FILE 'BIOSIS' ENTERED AT 10:16:02 ON 01 JAN 2008  
L11 82 S L10  
FILE 'MEDLINE' ENTERED AT 10:16:40 ON 01 JAN 2008  
L12 68 S L10  
FILE 'CA, BIOSIS, MEDLINE' ENTERED AT 10:17:19 ON 01 JAN 2008  
L13 113 DUP REM L10 L11 L12 (124 DUPLICATES REMOVED)

=> d bib,ab,kwic l13 1-113

L13 ANSWER 35 OF 113 CA COPYRIGHT 2008 ACS on STN  
AN 139:347654 CA  
TI **Near-membrane** iminocoumarin-based low affinity fluorescent Ca<sup>2+</sup>  
indicators  
AU Liepouri, F.; Deligeorgiev, T. G.; Veneti, Z.; Savakis, C.;  
Katerinopoulos, H. E.  
CS Department of Chemistry, University of Crete, Crete, 71 409, Greece  
SO Cell Calcium (2002), 31(5), 221-227  
AB Two new potential **near-membrane** iminocoumarin-based fluorescent Ca<sup>2+</sup>  
**indicators** were synthesized and the spectral profiles of their free and  
Ca<sup>2+</sup> bound forms were studied. The probes incorporate in their BAPTA-  
related structures, the 3-(benzimidazolyl)iminocoumarin or the 3-  
(benzothiazolyl)iminocoumarin moiety, substituted at the imino nitrogen  
with an n-dodecyl lipophilic chain. The compds. are excited with  
visible light and have Ca<sup>2+</sup> dissociation constants of 5.50 and 4.49 μM,  
resp., the highest reported to date in the literature. Fluorescence  
spectra studies indicated a clear shift in their excitation wavelength  
maxima upon Ca<sup>2+</sup> binding along with changes in fluorescence intensity  
that enable the compds. to be used as ratiometric **near-membrane**, low **Ca<sup>2+</sup>**  
affinity **probes**.

L13 ANSWER 66 OF 113 CA COPYRIGHT 2008 ACS on STN  
AN 125:52738 CA  
TI **Near-membrane** [Ca<sup>2+</sup>] transients resolved using the Ca<sup>2+</sup> indicator **FFP18**  
AU Etter, Elaine F.; Minta, Akwasi; Poenie, Martin; Fay, Fredric S.  
CS Dep. Physiology Biomedical Imaging Group, Univ. Massachusetts Med.

SO Center, orcester, MA, 01605, USA  
AB Proceedings of the National Academy of Sciences of the United States of America (1996), 93(11), 5368-5373  
AB Ca<sup>2+</sup>-sensitive processes at cell membranes involved in contraction, secretion, and neurotransmitter release are activated in situ or in vitro by Ca<sup>2+</sup> concns. [(Ca<sup>2+</sup>)] 10-100 times higher than [Ca<sup>2+</sup>] measured during stimulation in intact cells. This paradox might be explained if the local [Ca<sup>2+</sup>] at the cell membrane is very different from that in the rest of the cell. Sol. Ca<sup>2+</sup> indicators, which indicate spatially averaged cytoplasmic [Ca<sup>2+</sup>], cannot resolve these localized, **near-membrane** [Ca<sup>2+</sup>] signals. **FFP18**, the newest **Ca<sup>2+</sup> indicator** designed to selectively **monitor near-membrane** [Ca<sup>2+</sup>], has a lower Ca<sup>2+</sup> affinity and is more water sol. than previously used membrane-assocg. Ca<sup>2+</sup> indicators. Images of the intracellular distribution of **FFP18** show that >65% is located on or **near** the plasma **membrane**. [Ca<sup>2+</sup>] transients recorded using **FFP18** during membrane depolarization-induced Ca<sup>2+</sup> influx show that **near-membrane** [Ca<sup>2+</sup>] rises faster and reaches micromolar levels at early times when the cytoplasmic [Ca<sup>2+</sup>], recorded using fura-2, has risen to only a few hundred nanomolar. High-speed series of digital images of [Ca<sup>2+</sup>] show that **near-membrane** [Ca<sup>2+</sup>], **reported** by **FFP18**, rises within 20 ms, peaks at 50-100 ms, and then declines. [Ca<sup>2+</sup>] **reported** by fura-2 rose slowly and continuously throughout the time images were acquired. The existence of these large, rapid increases in [Ca<sup>2+</sup>] directly beneath the surface membrane may explain how numerous Ca<sup>2+</sup>-sensitive membrane processes are activated at times when bulk cytoplasmic [Ca<sup>2+</sup>] changes are too small to activate them.

L13 ANSWER 71 OF 113 CA COPYRIGHT 2008 ACS on STN  
AN 125:7754 CA  
TI **Near membrane** Ca<sup>2+</sup> changes resulting from store release in neutrophils: detection by **FFP-18**  
AU Davies, E. V.; Hallett, M. B.  
CS Mol. Signalling Group, Univ. Wales, Cardiff, UK  
SO Cell Calcium (1996), 19(4), 355-362  
AB **FFP-18** was incorporated into the inner face of the plasma membrane of human neutrophils by incubation with its acetoxyethyl ester. Conversion to the **Ca<sup>2+</sup>** sensitive intracellular **indicator** was **monitored** by the change in excitation spectra. The fluorescence from extracellularly facing **FFP-18** was quenched by the membrane impermeant ion Na<sup>+</sup>. Ratio fluorescence measurement of **FFP-18** under these conditions permitted the **detection** of **near membrane** Ca<sup>2+</sup> changes resulting from the release of Ca<sup>2+</sup> from intracellular stores. **Near membrane** and cytosolic **Ca<sup>2+</sup>** changes were **measured** under conditions in which store release and Ca<sup>2+</sup> influx were triggered by fMLP, thapsigargin or immune complexes. There were significant differences in the timing and magnitude of Ca<sup>2+</sup> storage site deep within the neutrophil released by thapsigargin and fMLP, but Ca<sup>2+</sup> near the inner face of the plasma membrane thus provides evidence for the existence of two distinct Ca<sup>2+</sup> storage locations in neutrophils.

L13 ANSWER 74 OF 113 CA COPYRIGHT 2008 ACS on STN  
AN 125:81153 CA

- TI Synthesis and characterization of leakage resistant and **near membrane** fluorescent calcium **indicator** dyes  
AU Vorndran, Charles  
CS Univ. of Texas, Austin, TX, USA  
SO (1995) 184 pp. Avail.: Univ. Microfilms Int., Order No. DA9617367 From:  
Diss. Abstr. Int., B 1996, 57(1), 62  
TI Synthesis and characterization of leakage resistant and **near membrane** fluorescent calcium **indicator** dyes
- L13 ANSWER 76 OF 113 CA COPYRIGHT 2008 ACS on STN  
AN 124:4271 CA  
TI New fluorescent calcium **indicators** designed for cytosolic retention or **measuring calcium near membranes**  
AU Vorndran, Charles; Minta, Akwasi; Poenie, Martin  
CS Dep. Zool., Univ. Texas, Austin, TX, 78712-1064, USA  
SO Biophysical Journal (1995), 69(5), 2112-24  
AB A new family of fluorescent calcium indicators has been developed based on a new analog of BAPTA called FF6. This new BAPTA analog serves as a versatile synthetic intermediate for developing Ca<sup>2+</sup> indicators targeted to specific intracellular environments. Two of these new Ca<sup>2+</sup> indicators, fura-PE3 and fura-**FFP18**, are described in this report. Fura-PE3 is a **zwitterionic** indicator that resists the rapid leakage and compartmentalization seen with fura-2 and other polycarboxylate calcium indicators. In contrast to results obtained with fura-2, cells loaded with PE3 remain brightly loaded and responsive to changes in concn. of cytosolic free calcium for hours. Fura-**FFP18** is an amphipathic indicator that binds to liposomes and to cell membranes. Studies to be detailed later indicate that **FFP18** functions as a **near-membrane** Ca<sup>2+</sup> **indicator** and that calcium levels **near** the plasma **membrane** rise faster and higher than in the cytosol.
- L13 ANSWER 82 OF 113 CA COPYRIGHT 2008 ACS on STN  
AN 120:293334 CA  
TI **Detection** of changes in **near-membrane** Ca<sup>2+</sup> concentration using a novel membrane-associated Ca<sup>2+</sup> indicator  
AU Etter, Elaine F.; Kuhn, Michael A.; Fay, Fredric S.  
CS Med. Sch., Univ. Massachusetts, Worcester, MA, 01605, USA  
SO Journal of Biological Chemistry (1994), 269(13), 10141-9  
AB A Ca<sup>2+</sup> indicator has been synthesized and characterized which can be used to **monitor** rapid changes in the free **Ca<sup>2+</sup>** concn. ([Ca<sup>2+</sup>]) immediately **adjacent** to cell **membranes**. This indicator, referred to as C18-Fura-2, consists of a Fura-2 mol. conjugated to a lipophilic alkyl chain which will insert into cell membranes. When assocd. with cell membranes in low concns., C18-Fura-2 exhibits an excitation spectrum with a large Stokes shift and a single isosbestic point, thus [Ca<sup>2+</sup>] can be calcd. ratiometrically. The apparent Ca<sup>2+</sup> dissocn. const. of cell-assocd. C18-Fura-2 is around 150 nM. C18-Fura-2 orients in the cell membrane so that the fluorophore is facing the side to which it was applied. C18-Fura-2 was used to record rapid changes in intracellular [Ca<sup>2+</sup>] which occurred in response to membrane depolarization in isolated smooth muscle cells. The initial rise of the [Ca<sup>2+</sup>] transient reported by C18-Fura-2 was four to six times faster than the rise of the [Ca<sup>2+</sup>] transient reported by cytosolic Fura-2. This result suggests that C18-

Fura-2 was located at the plasma membrane near sites of Ca<sup>2+</sup> influx and indicates that membrane-assocd. Ca<sup>2+</sup> indicators can be used to detect rapid, localized changes in [Ca<sup>2+</sup>] which are obscured in signals recorded using water-sol., bulk cytosolic fluorescent Ca<sup>2+</sup> indicators.

=> log y  
STN INTERNATIONAL LOGOFF AT 10:18:45 ON 01 JAN 2008

=> d his

(FILE 'HOME' ENTERED AT 06:10:52 ON 01 JAN 2008)  
FILE 'REGISTRY' ENTERED AT 06:11:10 ON 01 JAN 2008  
L1           STRUCTURE UPLOADED  
L2           STRUCTURE UPLOADED  
L3           0 S L1  
L4           1 S L2  
L5           20 S L2 FULL  
FILE 'CA' ENTERED AT 06:18:38 ON 01 JAN 2008  
L6           336 S L5  
L7           2340 S FLUO  
L8           74 S L7 AND DERIVATI?  
L9           301 S L6 AND(CALCIUM OR CA OR CA2)  
L10          8 S L9 AND LEAK?  
L11          80 S L9 AND MEMBRANE  
L12          27 S L6 AND DERIVATI?  
L13          93 S L6-7 AND MODIF?  
L14          240 S L8,L10-13  
L15          165 S L14 AND PY<2004  
L16          40 S L14 AND PY<2006 AND PATENT/DT  
L17          3 S L6 AND MINTA ?/AU  
L18          10 S L6-7(5A) (DERIVATI? OR MODIF?)  
L19          50 S L6-7(8A) (LEAK? OR MEMBRANE)  
L20          49 S L18-19 AND PY<2005  
FILE 'BIOSIS' ENTERED AT 06:36:43 ON 01 JAN 2008  
L21          50 S L20  
FILE 'MEDLINE' ENTERED AT 06:37:01 ON 01 JAN 2008  
L22          38 S L20  
FILE 'CA, BIOSIS, MEDLINE' ENTERED AT 06:38:45 ON 01 JAN 2008  
L23          216 DUP REM L15 L16 L17 L20 L21 L22 (129 DUPLICATES REMOVED)

=> d bib,ab,kwic 1-216 123

L23 ANSWER 16 OF 216 BIOSIS on STN  
AN 2004:288730 BIOSIS  
TI Near-Membrane Ca<sup>2+</sup> Measurement with Novel Fluorochromes in Arterial Myocytes.  
AU Cavalli, Maurizio [Reprint Author]; Lee, Moo Yeol; Ohkura, Masamichi; Song, Hong; Zhang, Jin; Kinsey, Stephen P; Nakai, Junichi; Kotlikoff, Michael I; Blaustein, Mordecai P  
CS Physiol, U Maryland Med Sch, 655 W. Baltimore St, Baltimore, MD, 21201, USA mcava001@umaryland.edu  
SO FASEB Journal, (2004) Vol. 18, No. 4-5, pp. Abst. 829.11.  
http://www.fasebj.org/. e-file. Meeting Info.: FASEB Meeting on Experimental Biology: Translating the Genome. Washington, District of

Columbia, USA. April 17-21, 2004. FASEB.

AB PlasmERosomes, Ca<sup>2+</sup> signaling complexes, consist of certain plasma membrane (PM) microdomains, the subjacent "junctional" sarco- (or endo-) plasmic reticulum, and the intervening cytosol. Ca<sup>2+</sup> concentrations in these tiny sub-PM cytosolic spaces ((Ca<sup>2+</sup>)<sub>SPM</sub>) are apparently regulated independently of the Ca<sup>2+</sup> in bulk cytosol. Novel "near-membrane" Ca<sup>2+</sup> indicators should enable us to measure (Ca<sup>2+</sup>)<sub>SPM</sub> and thereby study PlasmERosome function directly. Fluo-MOMO-AM (TefLabs, Austin, TX), a fluorochrome based on Fluo-4-AM, was loaded into intact rodent small mesenteric arteries (RSMA). Confocal microscopy verified that Fluo-MOMO is anchored to PM and organelle membranes by a hydrophobic tail, and that it detects cytosolic Ca<sup>2+</sup> signals. We also generated PM-targeted derivatives of G-CaMP, a Ca<sup>2+</sup>-sensitive dye based on green fluorescent protein (Nakai et al., Nature Biotech. 19:137, 2001). We fused the gene for an improved G-CaMP (G-CaMP2; with increased quantum efficiency and extinction coefficient) to the C-terminus of the gene for the Na<sup>+</sup> pump (1 subunit that is uniformly distributed in the PM. Plasmids were transfected into intact RSMA and primary cultured artery myocytes. Confocal and wide field imaging verified the PM localization of expressed protein and its ability to detect Ca<sup>2+</sup> signals. A gene for G-CaMP2 fused to the Na/Ca exchanger isoform 1 that is confined to PlasmERosomes was also constructed.

L23 ANSWER 200 OF 216 CA COPYRIGHT 2008 ACS on STN  
AN 112:135620 CA  
TI Preparation and properties of calcium-specific, long-wavelength indicator dyes  
IN Tsien, Roger Yonchien; Minta, Akwasi  
PA University of California, Berkeley, USA  
SO Eur. Pat. Appl., 27 pp.  
PI EP 314480 A2 19890503 EP 1988-310120 19881027  
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US 5049673 A 19910917 US 1987-115921 19871030  
<--  
PRAI US 1987-115921 A 19871030  
OS MARPAT 112:135620  
AB The title dyes I and II [E1, E2 = H, Me, Et, CH<sub>2</sub>OH, CO<sub>2</sub>H, CH<sub>2</sub>CO<sub>2</sub>H, or E1E2 = (CH<sub>2</sub>)<sub>m</sub>VCH<sub>2</sub>)<sub>n</sub> (sic; m, n = 1, 2; V = CH<sub>2</sub>, O, NH, NMe, S, SS); W = H, OH, CO<sub>2</sub>H; X = H, Me, CO<sub>2</sub>H, F, Cl, Br, I, NO<sub>2</sub>; Y = O, NMe, S, CH<sub>2</sub>, CMe<sub>2</sub>, CF<sub>2</sub>, C:O, bond; Z<sub>1</sub>, Z<sub>2</sub>, Z<sub>3</sub>, Z<sub>4</sub> = H, F, Cl, Br, I, Me; Q<sub>1</sub>, Q<sub>2</sub> = R<sub>1</sub>R<sub>2</sub>N, R<sub>1</sub>R<sub>2</sub>N:+, (R<sub>1</sub>, R<sub>2</sub> = H, Me, Et), OH-, O<sub>2</sub>-, etc.] and their pharmaceutically acceptable nontoxic salts and esters are provided. Binding of Ca<sup>2+</sup> increases the fluorescence of the above dyes by up to 40-fold. The Ca<sup>2+</sup> dissochn. consts. are in the range 0.37-2.3 .mu.M, so that the indicators give better resoln. of high Ca<sup>2+</sup> concns. than were previously obtainable with predecessor compds. The visible excitation wavelengths of I and II are more convenient for fluorescent microscopy and flow cytometry than the UV required by previous indicators. Thus, III was prep'd. from reaction of 2,7-dichloro-3,6-dihydroxyxanth-9-one and an organolithium deriv. (prepn. given) of 1-(2-aminophenoxy)-2-(2-amino-5-phenoxy)ethane, followed by removal of tert-Bu groups. After purifn., extinction coeffs. were 7.9 .times. 104 and 8.3 .times. 104 M<sup>-1</sup> cm<sup>-1</sup> at 503 and 506 nm, resp., for free and Ca-bound III. Excitation and emission max. for III in the presence of excess Ca were 506 and 526

nm, resp.; quantum efficiencies in the absence of Ca and in the presence of excess Ca were 0.0051 and 0.183, resp. The fluorescence ratio of III in excess Ca vs. no Ca was 36-40. The effective dissociation const. for Ca<sup>2+</sup> was 0.45 .mu.M.

L23 ANSWER 201 OF 216 CA COPYRIGHT 2008 ACS on STN DUPLICATE 73  
AN 111:53566 CA <<LOGINID::20080101>>  
TI Fluorescent indicators for cytosolic calcium based on rhodamine and fluorescein chromophores  
AU Minta, Akwasi; Kao, Joseph P. Y.; Tsien, Roger Y.  
CS Dep. Physiol.-Anat., Univ. California, Berkeley, CA, 94720, USA  
SO Journal of Biological Chemistry (1989), 264(14), 8171-8  
AB A new group of fluorescent indicators with visible excitation and emission wavelengths was synthesized for measurements of cytosolic free Ca<sup>2+</sup>. The 5 compds., rhod-1, rhod-2, fluo-1, fluo-2, and fluo-3, combine the 8-coordinate tetracarboxylate chelating site of 1,2-bis(2-amino-phenoxyethane-N,N,N',N'-tetraacetic acid with a xanthene chromophore to give a rhodamine-like or fluorescein-like fluorophore. Binding of Ca<sup>2+</sup> increases the fluorescence by up to 40-fold. The Ca<sup>2+</sup> dissociation constants are in the range 0.37-2.3 μM so that the new indicators should give better resolution of high [Ca<sup>2+</sup>] levels than previously obtainable with quin-2 or fura-2. The visible excitation wavelengths of the new compds. are more convenient for fluorescence microscopy and flow cytometry than the UV required by previous indicators. However, the increase in fluorescence of the new dye upon binding Ca is not accompanied by a wavelength shift, so they are unsuitable for measurements using ratios at 2 wavelengths. The most promising dye of this series is fluo-3, which was tested in fibroblasts.

=> log y

STN INTERNATIONAL LOGOFF AT 06:41:47 ON 01 JAN 2008